CERTIFICATE OF EXPRESS MAILING

EXPRESS MAIL Mailing Label Number: EL 889409921 US Date of Deposit July 30, 2003

I hereby certify that this correspondence, fee, and all accompanying papers, if any, are being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Francene Sawyer July 30, 2003

Francene Sawyer Date

U.S. Patent Application Entitled

USE OF ALKYLPHOSPHOCHOLINES IN COMBINATION WITH ANTITUMOR MEDICATIONS FOR THE TREATMENT OF BENIGN AND MALIGNANT ONCOSES IN HUMANS AND MAMMALS

Inventors: Jürgen Engel, Eckhard Günther, and Herbert Sindermann

TITLE

[0001] Use of Alkylphosphocholines in Combination with Antitumor Medications for the Treatment of Benign and Malignant Oncoses in Humans and Mammals

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application No. 60/399,615, filed July 30, 2002, which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0003] Alkylphosphocholines are a new class of organic compounds, which exhibit diversified anti-neoplastic activities (*M. Lohmeyer and R. Bittman*, Antitumor Ether Lipids and Alkylphosphocholines, DOF, 19 (11), 1021-103 7 (1994)). The effect of the alkylphosphocholines in this connection may be based on different, molecular and biochemical mechanisms, some of which take place on the level of the plasma membrane of the cell.

[0004] It is well known that alkylphosphocholines influence inositol metabolism, the interaction with phospholipases or inhibition of protein kinase C and thus that this class of substances has a general influence on cellular signal transduction (*K. Maly et al.*, Interference of New Alkylphospholipid Analogues With Mitogenic Signal Transduction, Anti-Cancer Drug Design, 10, 411-425 (1995); and *P. Hilgard, et al.*, D21266, A New Heterocyclic Alkylphospholipid with Antitumor Activity, Eur. J. Cancer, 33 (3), 442-446 (1997)). Thus, the alkylphosphocholine perifosine shows growth-inhibitory properties in relation to various

melanoma melanoma, CNS, lung, colon, prostate and breast cancer cell lines with an IC_{50} ranging from 0.2 to 20 μM.

[0005] It is further known that perifosine blocks tumor cells in the G_1-S and G_2-M phase of the cell cycle (*V. Patel, et al.*, A Novel Alkylphospholipid, Induces p.21 ^{Wafl} Expression in Squamous Carcinoma Cells through a p53-independent Pathway, Leading to Loss in Cyclindependent Kinase Activity and Cell Cycle Arrest, Cancer Research 62, 1401-1409 (2002)).

[0006] It is known that the use of alkylphosphocholines before or together with radiation therapy leads its synergistic effects during the treatment of tumors (*P. Principe et al.*, Evaluation of Combinations of Antineoplastic Ether Phospholipids and Chemotherapeutic Drugs, Anti-Cancer Drugs, 3 (6), 577-587 (1992)). It has also been reported that different glycerol-3-phospholipids, such as ET- 18-$OOCH_3$, in combination with different DNA-interacting substances or tubulin binders increase the anti-tumor activity in vitro in a different tumor cell lines (*P. Principe et al.*, Synergistic Cytotoxic Effect of Aza-alkylphospholipids in Association with Chemotherapeutic Drugs, J. Lipid Mediators Cell Signalling, 10 (1-2), 171-173 (1994)).

BRIEF SUMMARY OF THE INVENTION

[0007] Surprisingly, it was now possible to show that linear alkylphosphocholines of the general Formulas I and II are suitable for use in a combination according to the invention with other drug products for the treatment of benign and malignant oncoses in humans and mammals.

[0008] In this connection, the present invention relates to the novel use of alkylphosphocholine in combination with antitumor medications for art-recognized

therapeutic activities attributed to the treatment of benign and malignant oncoses in humans and mammals.

[0009] It is therefore an object of this invention to provide a novel means of treating tumors with an inventive combination of linear alkylphosphocholines and anti-tumor substances.

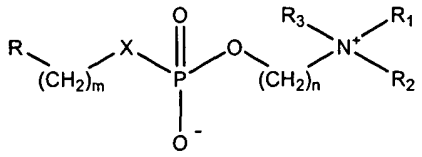
DETAILED DESCRIPTION OF THE INVENTION

[0010] The present invention provides a novel use of linear alkylphosphocholines of the general Formulas I and II in an inventive combination with other medicinal drugs for the treatment of benign and malignant oncoses in humans and mammals. According to one aspect of the invention, the compounds of the general Formulas I and II can be used in an inventive combination with anti-tumor substances. Anti-tumor substances may be alkylating agents, anti-metabolites, plant alkaloids, platinum compounds, tumor antibiotics and agonists or antagonists of natural hormones. The anti-tumor substances may be selected from, but are not restricted to cis-platinum, carboplatinum, oxaliplatinum, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, cyclophosphamide, 5-fluorouracil, fludarabin, gemcitabin and cytarabin.

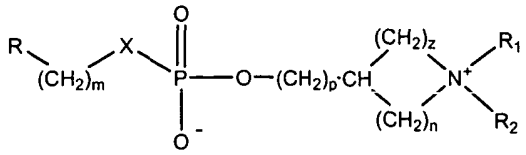
[0011] It is moreover possible for the alkylphosphocholines of the general Formula I and II to be employed in a claimed combination with inhibitors of signal transduction in the form of high and low molecular weight inhibitors of receptor and/or cytosolic kinases. These inhibitors may be selected from but not restricted to monoclonal antibodies and heterocyclic compounds.

[0012] The alkylphosphocholines of the general Formulas I and II, on which the invention is based, may be used in the form of finished medicinal drugs.

[0013] The compounds, on which the invention is based, are described by the general Formulas I and II:



Formula I



Formula II

in which, independently of one another,

n, m, p, z is a whole number between 0 and 4,

X is O, S, NH;

R is hydrogen, a linear or branched C_1 to C_{20} alkyl group, which may be saturated or unsaturated with one to three double and/or triple bonds and unsubstituted or optionally substituted at the same or at different carbon atoms with one, two or more halogen, nitro, cyano, hydroxy, C_1 to C_6 alkoxy, amino, mono-(C_1 to C_4) alkylamino or di-(C_1 to C_4) alkylamino groups,

R_1, R_2, R_3 independently of one another represent hydrogen, a linear or branched (C_1 to C_6) alkyl group, preferably methyl and ethyl, a (C_3 to CO cyclo alkyl group, which may be unsubstituted or optionally substituted at the same or different carbon atoms with one, two or more halogen, nitro, cyano, hydroxy, C_1 to C_6 alkoxy, amino, mono-(C_1 to C_4) alkylamino or di-(C_1 to C_4) alkylamino groups.

[0014] According to a further aspect of the invention, a method for controlling tumors in humans and in mammals is provided and comprises administering at least one of the compounds of the general Formula I and II on which the invention is based to the human or a mammal in an amount effective for tumor treatment before or during a treatment with approved antitumor substances.

[0015] The therapeutically effective dose, to be administered for the treatment, of the particular compound of the general formula I and II on which the invention is based depends inter alia on the nature and the stage of the oncosis, the age and sex of the patient, the mode of administration and the duration of treatment.

[0016] The compounds on which the invention are based can be administered in a drug product as liquid, semisolid and solid drug forms. This takes place in the manner suitable in each case in the form of aerosols, oral powders, dusting powders and epipastics, uncoated tablets, coated tablets, emulsions, foams, solutions, suspensions, gels, ointments, pastes, pills, pastilles, capsules or suppositories.

Examples:

[0017] **Example 1**. Administration of perifosine (D-21 266) in combination with cisplatin

In vivo Experiment: DMBA-induced rat mammary carcinoma model

Experimental Animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosin 14 x 6.81 mg/kg p.o.

Cis-platinum 4 x 1 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

TABLE 1

Treatment	Tumor Starting Weight (g)	Day 21, Change in %	p Test vs. Control
Control	1.0	875	--
Perifosin (D-21266)	0.9	-25	<0.001
Cis-platinum	0.9	410	0.120
Perifosin (D-21266) + Cis-platinum	0.8	-75	<0.001

[0018] **Example 2**. Administration of perifosine in combination with cyclophosphamide

In vivo Experiment: DMBA-induced rat mammary carcinoma model

Experimental Animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was

estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.

Cyclophosphamide 100 mg/kg, VZ 0, i.v.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

TABLE 2

Treatment	Tumor Starting Weight (g)	Day 21, Change in %	p Test vs. Control
Control	1.0	875	--
Perifosin (D-21266)	0.9	-25	<0.001
Cyclophosphamide	0.9	500	0.011
Perifosin (D-21266) + Cyclophosphamide	0.8	-83.3	<0.001

[0019] **Example 3**. Administration of perifosine in combination with adriamycin

In vivo Experiment: DMBA-induced rat mammary carcinoma model

Experimental Animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The eight of the tumor was mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.

Adriamycin 4 x 2.15 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

TABLE 3

Treatment	Tumor Starting Weight (g)	Day 21, Change in %	p Test vs. Control
Control	1.0	875	--
Perifosin (D-21266)	0.9	-25	<0.001
Adriamycin	1.0	781	0.197
Perifosin (D-21266) + Adriamycin	1.9	-70	<0.001

[0020] In the manner described above, the present invention thus provides a method for the use of alkylphosphochlorines in combination with antitumor medications for the treatment of benign and malignant oncoses in humans and mammals. While this invention has been described with reference to the preferred embodiments, these are illustrative only and not limiting, having been presented by way of example. Other modifications will become apparent to those skilled in the art by study of the specification and drawings. It is thus intended that the following appended claims include such modifications as fall within the spirit and scope of the present invention.

[0021] What we claim is: